Prostaglandin E (EP) receptor subtypes and sleep: promotion by EP_4 and inhibition by EP_1/EP_2

Yasushi Yoshida, Hitoshi Matsumura,[CA] Tomoko Nakajima, Masaharu Mandai, Takahito Urakami, Kenji Kuroda and Hiroshi Yoneda

Department of Neuropsychiatry, Osaka Medical College, 2-7 Daigakumachi, Takatsuki-City, Osaka 569-8686, Japan

[CA]Corresponding Author

Received 11 April 2000; accepted 20 April 2000

Prostaglandin (PG) E_2 reportedly augmented wakefulness when continuously infused into the third ventricle of the rat brain, whereas it promoted sleep when continuously infused into the subarachnoid space of the ventral surface zone of the rostral basal forebrain, which was designated previously as a PGD_2-sensitive sleep-promoting zone (PGD_2-SZ). In the present study, we investigated the effects of PGE (EP)-receptor agonists on sleep-wakefulness activities by infusing agonists into the third ventricle or into the subarachnoid space of the PGD_2-SZ. Our results indicated that the waking effect is mediated by EP_1 and EP_2 receptors situating around the third ventricle, whereas the sleep-promoting effect is brought about mainly through activation of EP_4 receptors located at or near the subarachnoid space of the PGD_2-SZ. *NeuroReport* 11:2127–2131 © 2000 Lippincott Williams & Wilkins.

Key words: EP receptor subtype; Prostaglandin D_2-sensitive sleep-promoting zone; Prostaglandin E_2; Sleep–wakefulness regulation

INTRODUCTION

Prostaglandin (PG) D_2 and PGE_2, major biologically active eicosanoids in the mammalian brain, have been investigated vigorously in rats, mice, monkeys, and other animals for their effects on sleep–wakefulness activities [1–8]. For example, PGE_2 continuously infused into the third ventricle augmented wakefulness and suppressed both slow-wave sleep (SWS) and paradoxical sleep (PS) in freely moving rats [4,5]. In contrast, PGD_2 was found to exhibit a sleep-promoting effect in rats [1] and monkeys [3]. The site of action of PGD_2 for its effect of promoting sleep was subsequently demarcated in the ventral surface zone of the rostral basal forebrain, which was designated as a PGD_2-sensitive sleep-promoting zone (PGD_2-SZ) [7]. A more recent study showed that not only PGD_2 but also PGE_2 and $PGF_2\alpha$ promoted sleep when continuously infused into the subarachnoid space of the PGD_2-SZ during the nocturnal hours and that the SWS-promoting effects of PGE_2 and $PGF_{2\alpha}$ were almost equipotent to that effect of PGD_2 [8]. It is intriguing to note that PGE_2 has two effects in the opposite direction; i.e. wakefulness-augmenting and sleep-promoting effects, which are exhibited by acting around the third ventricle and in the PGD_2-SZ, respectively.

PGE_2 has a variety of central actions, including generation of fever [9], nociceptive behavior [10] and neuroendocrine function [11] as well as sleep–wakefulness regulation. The actions of PGE_2 are mediated by specific cell receptors; and according to pharmacological findings, the PGE (EP) receptor has been divided at least into four subtypes: EP_1, EP_2, EP_3 and EP_4 [12]. These EP receptor subtypes were cloned from humans, mice (for review, see [13]), and rats [14]. Signal transduction pathways for the respective EP receptors were reported as being associated with various kinds of G proteins [13]. Thus, it is inferred that the effects of PGE_2 on sleep–wakefulness activities depend on EP receptor subtypes that are expressed in proper regions of the rat brain.

AH6809, an EP_1/EP_2 antagonist, inhibited the waking effect of PGE_2 [5], thus suggesting that this effect of PGE_2 was mediated by EP_1 and/or EP_2 receptors. However, it is still unknown which subtype of the EP receptor is responsible for the sleep-promoting effect of PGE_2 administered to the PGD_2-SZ. In the present study, we investigated the effects of novel agonists [15] specific for respective EP receptor subtypes on sleep–wakefulness activities by infusing them into the third ventricle or into the subarachnoid space of the PGD_2-SZ.

MATERIALS AND METHODS

Adult male Sprague–Dawley rats (270–350 g, $n = 85$; Japan SLC, Hamamatsu City, Japan) were used in this study. The experimental environment was conditioned at a temperature of 25°C and 60% relative humidity under a 12:12 h light:dark (lights on 08:00–20:00 h) cycle. The animals were maintained under these conditions for about 7 days prior

to surgical operation, and were allowed free access to food and water.

Under pentobarbital anesthesia (50 mg/kg, i. p.), the rats underwent surgery for implantation of electrodes for recording electroencephalogram (EEG), electro-oculogram (EOG), and electromyogram (EMG). In rats that were to receive an infusion of an agonist into the third ventricle, one stainless-steel cannula (0.35 mm o.d.) was inserted from the point 0.8 mm posterior to bregma and 1.4 mm lateral to the midline to a depth of 7.8 mm from the level of bregma at an angle of 10° from the midsagittal plane, so that the tip of the cannula would be situated in the third ventricle. For infusion into the PGD_2-SZ, a pair of cannulae was introduced bilaterally from a point 1.7 mm anterior to bregma and 2.0 mm lateral to the midline with a depth of 8.9 mm at an angle of 10° from the midsagittal plane, so that their tips would be positioned in the subarachnoid space of the PGD_2-SZ. These implantation procedures were made according to the stereotaxic coordinates adopted from the rat brain atlas of Paxinos and Watson [16].

These animals were allowed to recover postoperatively for at least 9 days. Then they were moved into our originally devised experimental cages [17], where continuous infusion of vehicle solution (saline) through each cannula was commenced at a rate of 0.2 µl/min. After a period of 4 days for acclimation to this continuous infusion and other factors of the experimental milieu, recordings of EEG, EOG, and EMG were started. These recordings continued for at least 48 h, with the initial 24 h period defined as the baseline day and the second as the experimental day.

In the first series of experiments, infusion of vehicle through the single cannula into the third ventricle was changed to the infusion of a test solution containing an agonist between 11:00 h and 17:00 h (rest phase of the animal) of the experimental day to determine the subtype of the EP receptor that participated in the suppression of sleep or in the augmentation of wakefulness. In the second series of experiments, we switched the infusion of vehicle into the subarachnoid space of the PGD_2-SZ through the paired cannulae to the continuous infusion of a test solution containing an agonist between 23:00 h and 05:00 h (active phase) of the experimental day to determine the subtype that was involved in the promotion of sleep. The infusion of the EP_4 agonist into the subarachnoid space of the PGD_2-SZ was performed also between 11:00 and 17:00 h, as described in Results and Discussion.

After experiments, animals were sacrificed with an overdose of pentobarbital sodium, and injected with pontamine sky blue dye (0.5% w/v) through the implanted cannulae. Then, the brains were removed and fixed in 10% formalin, and the site of the tip of each cannula and location of its track were verified histologically.

On the EEG, EOG, and EMG recordings, SWS, PS, and wakefulness were scored based on visual determination. Minimal scoring interval was set at 15 s. The hourly amounts of SWS, PS, and wakefulness were calculated; and comparison was made between baseline and experimental data by use of paired Student's *t*-test. Data are expressed as mean ± s.e.m.

The EP receptor agonists used in this study, i.e. ONO-DI-004, ONO-AE1-257, ONO-AE-248 and ONO-AE1-329, are recently created highly selective EP_1, EP_2, EP_3 and EP_4 agonists, respectively [15], and were generous gifts from ONO Pharmaceutical Co., Ltd. (Osaka, Japan), of which ONO-AE1-257 is an EP_2 agonist possessing structure and agonist potency similar to those of the recently reported ONO-AE1-259 [15]. The compound ONO-DI-004, which was supplied in crystalline form, was dissolved in 100% dimethyl sulfoxide (DMSO) and diluted with saline solution before use. The final concentration of DMSO in the test solution was 5% (v/v). Other agonists were supplied in solutions at a concentration of 1 mg/ml: ONO-AE1-257 was in saline containing 4% sodium bicarbonate, whereas ONO-AE-248 and ONO-AE1-329 were in saline containing 0.5% ethanol and 1% Tween 80 and in saline containing 0.3% ethanol and 0.1% Tween 80, respectively. They were diluted with saline before use. The final concentrations of sodium bicarbonate, ethanol, and Tween 80 in the test solutions were 0.8%, 0.07–0.09%, and 0.02–0.2% (v/v), respectively. The saline solutions containing these solvents at the doses exhibited no marked changes in sleep–wakefulness activities in experiments performed in the same manner as described above (data not shown).

RESULTS

Effects of agonists infused into the third ventricle: Each of the agonists was infused continuously through each cannula at a rate of 100 pmol/0.2 µl/min except for the EP_1 agonist, which was tested only at the infusion rate of 10 pmol/0.2 µl/min due to its poor solubility. The infusion of EP_1 agonist into the third ventricle during diurnal hours (between 11:00 h and 17:00 h) significantly decreased the amount of SWS and increased wakefulness (Fig. 1a): the total SWS decrement during the 6 h infusion was 17 ± 4 min (baseline 231 ± 5 min; EP_1 agonist, 214 ± 4 min; $n = 10$; $p < 0.01$, paired *t*-test). The EP_2 agonist decreased PS (baseline, 42 ± 4 min; EP_2 agonist, 35 ± 2 min; $n = 8$; $p < 0.05$, paired *t*-test) and increased wakefulness. The EP_3 agonist produced no significant changes in SWS or PS or wakefulness. The EP_4 agonist significantly increased SWS and decreased PS.

Effects of agonists infused into the subarachnoid space of the PGD_2-SZ: With the 6 h infusion (between 23:00 h and 05:00 h) performed bilaterally at the infusion rate of 100 pmol/min per each cannula into the subarachnoid space of the PGD_2-SZ, the EP_2, EP_3 and EP_4 agonists significantly increased the amount of SWS (Fig. 1b). The EP_4 agonist, in particular, exhibited an extraordinarily potent effect: the total SWS increment during the 6 h infusion was 131 ± 9 min (baseline 126 ± 5 min; EP_4 agonist 257 ± 8 min; $n = 8$; $p < 0.001$). Infusion of the EP_2 agonist was also noticed to markedly increase PS. The amount of wakefulness was decreased by the EP_2, EP_3 and EP_4 agonists. The EP_1 agonist at 10 pmol/min exhibited no significant effects.

As shown in the 24 h profiles for the effects of the EP_4 agonist infused at 100 pmol/min (Fig. 2), SWS began to increase from the first hour of the infusion period; and the latency to the increase was calculated as < 30 min after the commencement of the agonist infusion. The significant increases in the hourly amount of SWS continued until 1 h after the cessation of the agonist infusion. The levels of the

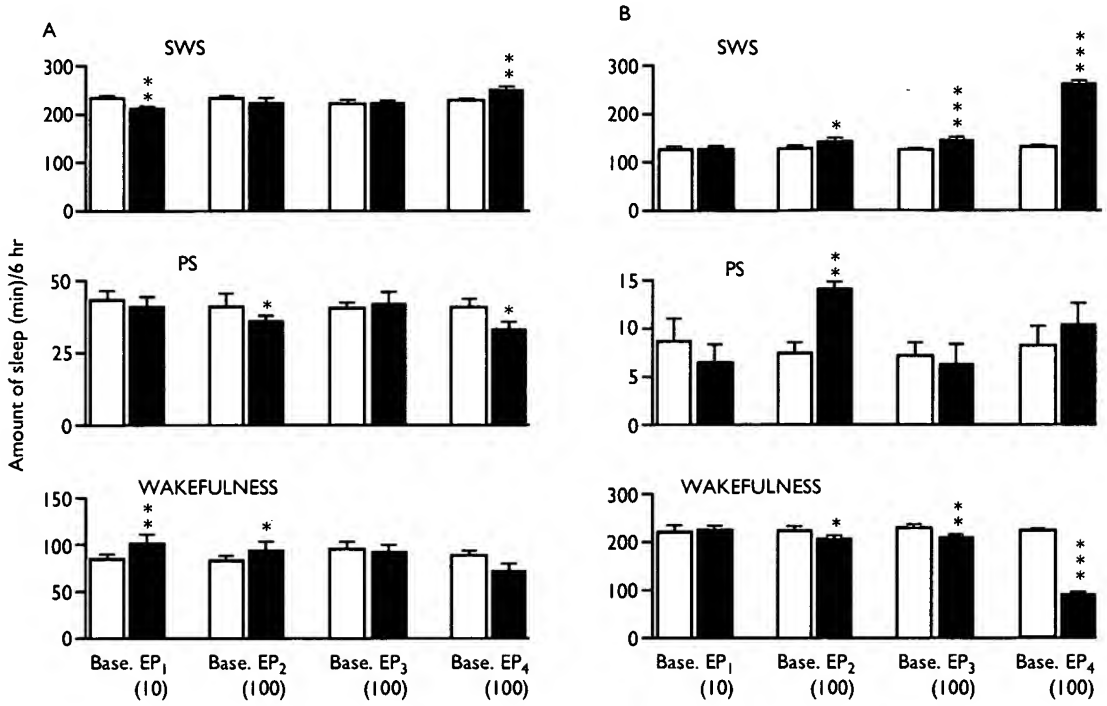

Fig. 1. Effects on SWS, PS, and wakefulness of EP agonists continuously infused into the third ventricle between 11:00 and 17:00 h (a) or into the subarachnoid space of the PGD_2-SZ between 23:00 h and 05:00 h (b). Each datum column represents the mean ± s.e.m. of 8–10 rats. Open and closed columns stand for baseline (Base) and experimental (EP) results, respectively. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by paired *t*-test for comparison between the experimental value and its corresponding baseline. The infusion of the agonists was made continuously at 100 pmol/min except for the EP_1 agonist, which was infused only at 10 pmol/min due to its poor solubility; and these infusion rates are indicated in parentheses below EP.

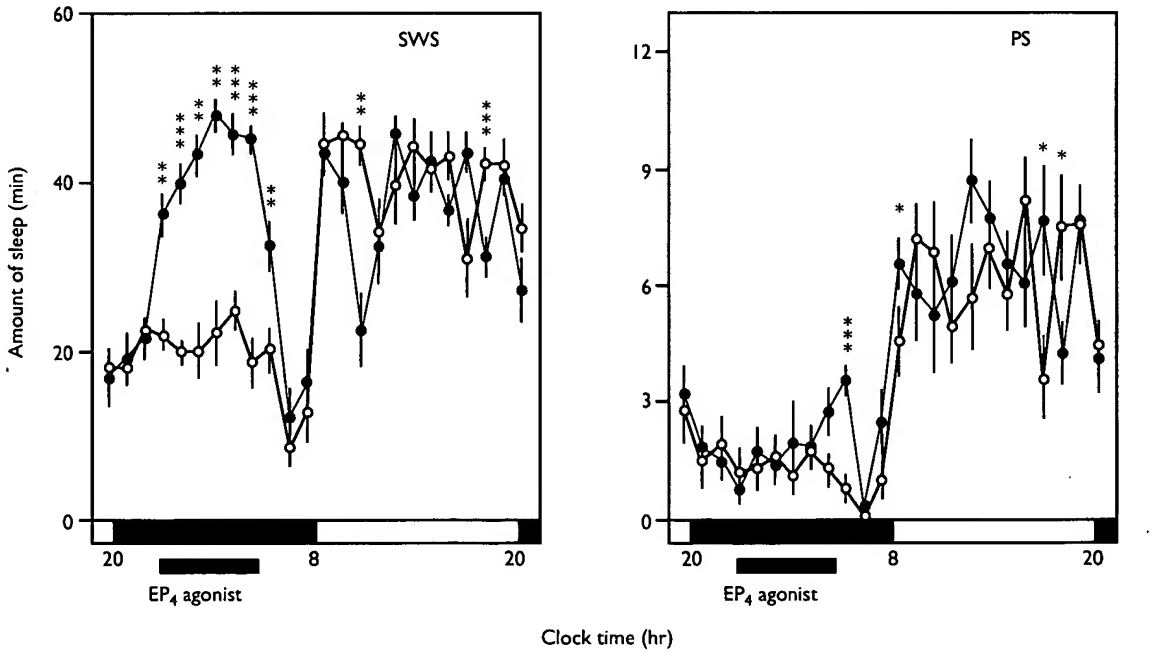

Fig. 2. Twenty-four hour profiles of SWS and PS of the animals (n=8), that received continuous bilateral infusion of the EP_4 agonist into the subarachnoid space of the PGD_2-SZ during the period indicated by the horizontal bars (23:00 h to 0500 h) on the experimental day at 100 pmol/min per side. Each datum point (open circles, baseline day; closed circles, experimental day) represents the hourly amount (mean ± s.e.m.) of SWS or PS. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by paired *t*-test for comparison between the experimental value and its corresponding baseline.

hourly amount of SWS during this 7 h period of SWS increase were comparable to those for the diurnal hours on the baseline day; thus, these hourly amounts, especially those during the latter half of the infusion period, attained the physiological maximum. A relatively small rebound decrease was seen during the daytime following the infusion period. As for PS, no significant change was observed during the infusion period, but a significant increase was transiently seen after cessation of the infusion of the agonist.

The total amount of SWS during the 6 h infusion of the EP_4 agonist increased in a dose-dependent manner at 10 and 100 pmol/min, whereas increases in PS became statistically significant at 1 and 10 pmol/min (Fig. 3).

The increase in SWS at 100 pmol/min was accompanied by prolongation (baseline 2.60 ± 0.32 min; EP_4 agonist 4.06 ± 0.46 min; $n = 8$; $p < 0.05$) and increased number of episodes (baseline 52 ± 4; EP_4 agonist 69 ± 7; $p < 0.05$). The marked increase in PS at 10 pmol/min was attributed to the increased number of PS episodes (baseline 7 ± 1; EP_4 agonist 14 ± 2; $n = 7$; $p < 0.01$), whereas the mean duration of the PS episodes was not significantly changed (baseline 1.34 ± 0.11 min; EP_4 agonist 1.43 ± 0.13 min).

Effect of the EP_4 agonist infused during the diurnal hours: We also tested the effect of the infusion of the EP_4 agonist into the PGD_2-SZ during the diurnal hours (between 11:00 h and 17:00 h). The EP_4 agonist at 100 pmol/min per cannula significantly increased SWS (baseline 232 ± 12 min; EP_4 agonist 273 ± 7 min; $n = 5$; $p < 0.01$) and decreased the amount of wakefulness (baseline 86 ± 16 min; EP_4 agonist 47 ± 7 min; $p < 0.05$), whereas the change in PS indicated no statistical significance (baseline 42 ± 4 min; EP_4 agonist 38 ± 3 min).

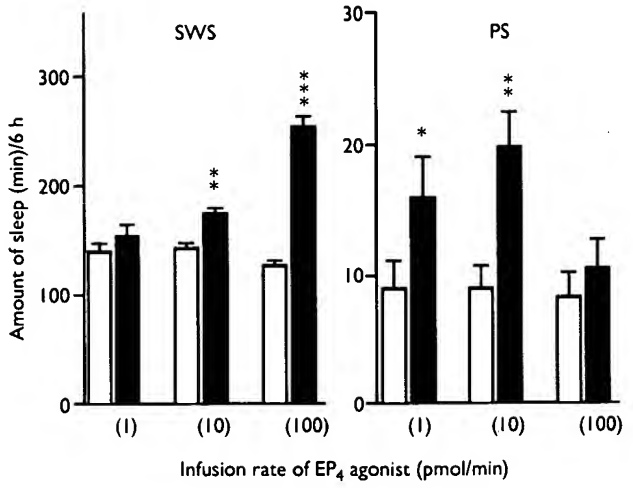

Fig. 3. Dose-response relationship for the EP_4 agonist, which was continuously infused into the subarachnoid space of the PGD_2-SZ between 23:00 h and 05:00 h. Each datum column represents the mean ± s.e.m. of 7–8 rats. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ by paired *t*-test for comparison between the experimental value (filled column) and its corresponding baseline (open column).

DISCUSSION

The continuous infusion into the third ventricle of the EP_1 and EP_2 agonists significantly decreased SWS and PS, respectively, suggesting that the waking- or sleep-inhibiting effect of PGE_2 is mediated by simultaneous activation of the two EP receptor subtypes, wherein activation of EP_1 receptors and that of EP_2 receptors may play primary roles in inhibiting SWS and PS, respectively. This postulation is also consistent with a previous finding that an infusion into the third ventricle of AH6809, an antagonist at EP_1/EP_2 receptors, increased both SWS and PS as well as inhibited the PGE_2-produced decreases in SWS and PS [5].

We infer that the site of action for the waking effects of the EP_1 and the EP_2 agonists is located in a diencephalic region based on the following findings: First, the SWS/PS inhibition by EP_1/EP_2 agonists was demonstrated with their infusion into the third ventricle but not with that into the subarachnoid space of the PGD_2-SZ in the present study; second, infusion of PGE_2 into the third ventricle of rats exhibited a waking effect [4,5], whereas that into the subarachnoid space of the PGD_2-SZ did not [8]; third, administration of PGE_2 to the hypothalamus increased wakefulness in monkeys [6] as well as in rats [2]; fourth, the level of PGE_2 in the hypothalamus was shown to be closely correlated with vigilance states in rats [18], e.g. the average concentration of PGE_2 during wakefulness was higher than that during SWS, and, in the middle of wakefulness, a drop after the increase in the slope of the PGE_2 curve predicted the occurrence of SWS; and finally mRNAs for EP_1- and EP_2-receptors were detected in various diencephalic regions of the brains of mice and rats [19,20].

At the PGD_2-SZ, infusion of the EP_4 agonist at 100 pmol/min during the nocturnal hours increased the amount of SWS, and the magnitude of this effect was extraordinarily potent. This profound increase in SWS was accompanied by prolongation and increased number of SWS episodes. Accordingly, the appearance of the SWS became similar to the one normally seen during diurnal hours in the rat. Thus, the present result suggested that the sleep-promoting effect of PGE_2 at the PGD_2-SZ is derived mainly from activation of the EP_4 receptors. Since a marked increase in SWS was also observed by the infusion during diurnal hours, the SWS-promoting effect of this agonist is thought to be independent of the influence of the circadian biological clock.

Continuous infusion of PGD_2 and that of $PGF_{2\alpha}$ into the subarachnoid space of the PGD_2-SZ during the nocturnal hours also promoted SWS [8]. The magnitude of the effect of the EP_4 agonist (100 pmol/min) at this site was comparable to the previously demonstrated effect of PGD_2 [7]. In the 24 h profile for SWS (Fig. 2), the latency to the SWS increase after the commencement of the agonist infusion was < 30 min, which was also comparable to or even shorter than that for PGD_2 found in our previous study [7]. A relatively small rebound decrease was seen in SWS during the daytime following the agonist infusion, and a significant PS increase was only transiently seen after cessation of the infusion. These features are also similar to those seen with the PGD_2 infusion. Thus, it might be conceivable that PGD_2/EP_4 agonist could be cross-reactive with PGD (DP)/EP_4 receptors with regard to the SWS-

promoting effect. However, PGD_2 was reported to have low affinity for the EP_4 receptor [14,21], and both PGE_2 and the EP_4 agonist were demonstrated to have marginal affinity for the DP receptor [15]. Therefore, we postulate that PGD_2 and PGE_2 promote sleep at or near PGD_2-SZ through activation of DP and EP_4 receptors, respectively.

In the present study, the EP_2 and EP_3 agonists also increased SWS significantly, although the magnitude was very small when compared with that for the EP_4 agonist. The small SWS increase combined with the large PS increase observed with infusion of the EP_2 agonist at 100 pmol/min (Fig. 1b) was similar to the SWS- and PS changes shown with infusion of the EP_4 agonist at lower doses (Fig. 3). Therefore, the EP_2-mediated regulation of sleep and the EP_4-mediated one may have some overlapping in the regulatory mechanisms in the rostral basal forebrain, notwithstanding the fact that the reported distribution patterns of the mRNAs for these two receptors in the rat brain were quite different from each other [20]. On the other hand, the EP_3 receptor was implicated in fever generation [22]. Whether the SWS increase is a phenomenon correlated with the EP_3-mediated hyperthermia or not remains to be further examined.

As for PGD_2, the mRNAs for both PGD synthase [23] and the DP receptor [24] were demonstrated in the leptomeninges of the rat brain; hence, it was supposed that the signals initiated in the subarachnoid space of the PGD_2-SZ were somehow transmitted to sleep-regulating neuronal systems in the brain parenchyma, which might include, at least in part, an activation of the adenosine A_{2A} receptors [25]. When taking into consideration the report [20] that the mRNA for the EP_4 receptor was present in the meninges of the rat brain, it might be considered that the membranous tissues in the PGD_2-SZ might also play a role in the promotion of sleep caused by the EP_4 agonist. However, different from the case for the DP receptor, the ventral septal/anterior preoptic areas and other basal forebrain structures exhibited moderate to strong signals for the EP_4-receptor mRNA under the basal condition in the rat [20]; therefore, it is also feasible that PGE_2 directly affects some groups of neurons in such structures through still undefined mechanisms to promote sleep.

CONCLUSIONS

PGE_2 has two effects in the opposite direction, i.e. a waking effect [2,4–6] and a sleep-promoting one [8]. Our results indicated that the waking effect of PGE_2 is primarily brought about through EP_1-receptor-mediated suppression of SWS and EP_2-receptor-mediated suppression of PS. The receptors implicated in these effects are supposedly situated in the diencephalon around the third ventricle. In contrast, other results of ours indicated that activation of the EP_4 receptors located at or near the PGD_2-SZ, which was defined previously in the ventral surface zone of the rostral basal forebrain [7], mediate the sleep promotion caused by PGE_2. The SWS increment demonstrated with the infusion of the EP_4 agonist into the PGD_2-SZ at the highest dose tested was extraordinarily large such that the magnitude was comparable to the previously demonstrated effect of PGD_2 [7]. Infusion of the EP_4 agonist at lower doses also increased PS markedly.

REFERENCES

1. Ueno R, Honda K, Inoué S *et al. Proc Natl Acad Sci USA* **80**, 1735–1737 (1983).
2. Matsumura H, Goh Y, Ueno R *et al. Brain Res* **444**, 265–272 (1988).
3. Onoe H, Ueno R, Fujita I *et al. Proc Natl Acad Sci USA* **85**, 4082–4086 (1988).
4. Matsumura H, Honda K, Goh Y *et al. Brain Res* **481**, 242–249 (1989).
5. Matsumura H, Honda K, Choi WS *et al. Proc Natl Acad Sci USA* **86**, 5666–5669 (1989).
6. Onoe H, Watanabe Y, Ono K *et al. J Neurosci* **12**, 2715–2725 (1992).
7. Matsumura H, Nakajima T, Osaka T *et al. Proc Natl Acad Sci USA* **91**, 11998–12002 (1994).
8. Ram A, Pandey HP, Matsumura H *et al. Brain Res* **751**, 81–89 (1997).
9. Milton AS and Wendlandt S. *J Physiol (Lond.)* **218**, 325–336 (1971).
10. Oka T, Aou S and Hori T. *Brain Res* **663**, 287–292 (1994).
11. Lacroix S, Vallieres L and Rivest S. J Neuroimmunol 70, 163-179 (1996).
12. Coleman RA, Grix SP, Head JB *et al. Prostaglandins* **47**, 151–168 (1994).
13. Ushikubi F, Hirata M and Narumiya S. *J Lipid Mediat* **12**, 343–359 (1995).
14. Boie Y, Stocco R, Sawyer N *et al. Eur J Pharmacol* **340**, 227–241 (1997).
15. Yamamoto H, Maruyama T, Sakata K *et al.* The 6th International Eicosanoid Conference, The Westin, Boston, MA. Abstract 152 (1999).
16. Paxinos G and Watson C. *The Rat Brain in Stereotaxic Coordinates.* San Diego: Academic Press; 1986.
17. Matsumura H, Kinoshita G, Satoh S *et al. J Neurosci Methods* **57**, 145–149 (1995).
18. Gerozissis K, de Saint Hilaire Z, Orosco M *et al. Brain Res* **689**, 239–244 (1995).
19. Batshake B, Nilsson C and Sundelin J. *Eur J Biochem* **231**, 809–814 (1995).
20. Zhang J and Rivest S. *Eur J Neurosci* **11**, 2651–2668 (1999).
21. Kiriyama M, Ushikubi F, Kobayashi T *et al. Br J Pharmacol* **122**, 217–224 (1997).
22. Ushikubi F, Segi E, Sugimoto Y *et al. Nature* **395**, 281–284 (1998).
23. Urade Y, Kitahama K, Ohishi H *et al. Proc Natl Acad Sci USA* **90**, 9070–9074 (1993).
24. Gerashchenko D, Beuckmann C, Kanaoka Y *et al. J Neurochem* **71**, 937–945 (1998).
25. Satoh S, Matsumura H, Suzuki F *et al. Proc Natl Acad Sci USA* **93**, 5980–5984 (1996).

Acknowledgements: We thank Drs Shinsuke Satoh (Kinki University School of Medicine) and Takashi Kanbayashi (Akita University) for valuable discussions. We are also grateful to Ms Urara Hayashi, Ms Yo Takada, Ms Yoshiko Takehara, and Ms Miyoko Kiyota for their excellent technical assistance.